REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978
APPLICATION FOR A PATENT AND
ACKNOWLEDGEMENT OF RECEIPT
(Section 30(1) Regulation 22)

FORM P.1
(to be lodged in duplicate)

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THE GRANT OF A PATENT IS HEREBY REQUESTED BY THE UNDERMENTIONED APPLICANT ON THE BASIS OF THE PRESENT APPLICATION FILED IN DUPLICATE.

PATENT APPLICATION NO.		
21	01	902839
71	FULL NAME(S) OF APPLICANT(S)	

A & A REF: 120193

AMERICAN CYANAMID COMPANY

ADDRESS(ES) OF APPLICANT(S)

ONE CYANAMID PLAZA, WAYNE, NEW JERSEY, U S A

54 TITLE OF INVENTION

N-ACYL DERIVATIVES OF THE LL-E33288 ANTITUMOR ANTIBIOTICS

Only the items marked with an "X" in the blocks below are applicable.

- [X] THE APPLICANT CLAIMS PRIORITY AS SET OUT ON THE ACCOMPANYING FORM P.2
- [ ] THE APPLICATION IS FOR A PATENT OF ADDITION TO PATENT APPLICATION NO. 21 01
- [ ] THIS APPLICATION IS A FRESH APPLICATION IN TERMS OF SECTION 37 AND BASED ON APPLICATION NO. 21 01

THIS APPLICATION IS ACCOMPANIED BY:

- [X] 1. ~~A single copy of a provisional specification~~ two copies of a complete specification of .....40... pages
- [X] 2. Drawings of ..8..... sheets. (GRAPHS)
- [X] 3. Publication particulars and abstract (Form P.8 in duplicate) (for complete only).
- [ ] 4. A copy of Figure .... of the drawings (if any) for the abstract (for complete only).
- [X] 5. An assignment of invention.
- [ ] 6. Certified priority document(s) (State quantity) : .....
- [ ] 7. Translation of the priority document(s).
- [ ] 8. An assignment of priority rights.
- [ ] 9. A copy of the Form P.2 and the specification of S.A. Patent Application No. 21 01
- [X] 10. A Form P.2 in duplicate.
- [ ] 11. A declaration and power of attorney on Form P.3.
- [ ] 12. Request for ante-dating on Form P.4.
- [ ] 13. Request for classification on Form P.9.
- [ ] 14. Request for delay of acceptance on Form P.4.
- [ ] 15.

74 ADDRESS FOR SERVICE: Adams & Adams, Pretoria.

DATED THIS 12 DAY OF APRIL 1990

.....
ADAMS & ADAMS
APPLICANTS PATENT ATTORNEYS

The duplicate will be returned to the applicant's address for service as proof of lodging but is not valid unless endorsed with official stamp.

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FORM P7

REPUBLIC OF SOUTH AFRICA
Patents Act, 1978

COMPLETE SPECIFICATION

(Section 30 (1) - Regulation 28)

OFFICIAL APPLICATION NO.	LODGING DATE
21 01 902839	22 12 APRIL 1990

INTERNATIONAL CLASSIFICATION

51 A61K, C07H

FULL NAMES(S) OF APPLICANT(S)

71 AMERICAN CYANAMID COMPANY

FULL NAME(S) OF INVENTOR(S)

72 MAY DEAN-MING LEE

TITLE OF INVENTION

54 N-ACYL DERIVATIVES OF THE LL-E33288 ANTITUMOR ANTIBIOTICS

~~[illegible]~~

Title: N-ACYL DERIVATIVES OF THE LL-E33288 ANTITUMOR ANTIBIOTICS

~~This is a continuation-in-part application of copending Serial No. 004,154, filed January 30, 1987.~~

SUMMARY OF THE INVENTION

The invention describes the N-acyl derivatives of the α_2^{Br}, β_1^{Br}, γ_1^{Br}, α_2^{I}, β_1^{I}, γ_1^{I}, and δ_1^{I} components and of the N-acyl-dihydro derivatives of the α_2^{Br}, β_1^{Br}, γ_1^{Br}, α_2^{I}, β_1^{I}, γ_1^{I}, and δ_1^{I} components of the LL-E33288 antibiotic complex prepared by reacting the antibiotic with an unsubstituted or substituted acid anhydride acyl cation equivalent or acid chloride. These N-acyl derivatives are effective antibacterial and antitumor agents.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure I: The proton magnetic resonance spectrum of N-acetyl-LL-E33288δ_1^{I}.

Figure II: The proton magnetic resonance spectrum of N-formyl-LL-E33288δ_1^{I}.

Figure III: The ultraviolet absorption spectrum of N-acetyl-LL-E33288γ_1^{I}.

Figure IV: The infrared absorption spectrum of N-acetyl-LL-E33288γ_1^{I}.

Figure V: The proton magnetic resonance spectrum of N-acetyl-LL-E33288γ_1^{I}.

Figure VI: The carbon-13 magnetic resonance spectrum of N-acetyl-LL-E33288γ_1^{I}.

Figure VII: The ultraviolet absorption spectrum of N-acetyl-dihydro-LL-E33288γ_1^I.

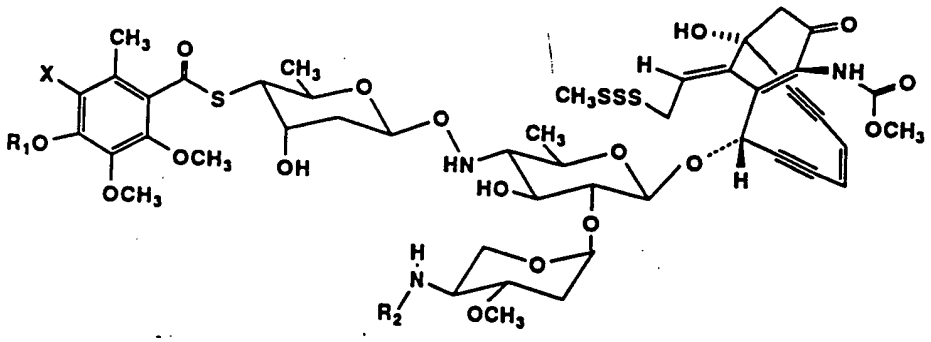
Figure VIII: The proton magnetic resonance spectrum of N-acetyl-dihydro-LL-E33288γ_1^I.

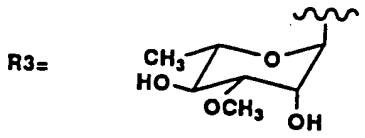
DETAILED DESCRIPTION OF THE INVENTION

The family of antibacterial and antitumor agents, known collectively as the LL-E33288 complex, are described and claimed in copending U.S. Patent Application Serial No. 009,321, filed January 30, 1987 and are used to prepare the N-acyl derivatives of this invention. The above application describes the LL-E33288 complex, the components thereof, namely, LL-E33288α_1^{Br}, LL-E33288α_2^{Br}, LL-E33288α_3^{Br}, LL-E33288α_4^{Br}, LL-E33288β_1^{Br}, LL-E33288β_2^{Br}, LL-E33288γ_1^{Br}, LL-E33288α_1^I, LL-E33288α_2^I, LL-E33288α_3^I, LL-E33288β_1^I, LL-E33288β_2^I, LL-E33288γ_1^I, and LL-E33288δ_1^I, and methods for their production by aerobic fermentation utilizing a new strain of Micromonospora echinospora ssp. calichensis or natural or derived mutants thereof. The proposed chemical structures of some of the above named components are disclosed in Serial No. 009,321 and are reproduced in Table I below.

Table I

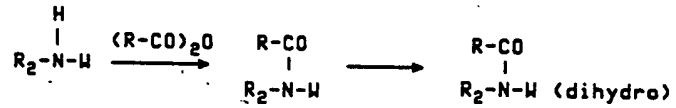
Proposed Structures for the LL-E33288 Components





Designation	R_1	R_2	X
E33288α_2^I	H	C_2H_5	I
E33288β_1^I	R_3	$(CH_3)_2CH$	I
E33288γ_1^I	R_3	C_2H_5	I
E33288δ_1^I	R_3	CH_3	I
E33288α_2^{Br}	H	C_2H_5	Br
E33288β_1^{Br}	R_3	$(CH_3)_2CH$	Br
E33288γ_1^{Br}	R_3	C_2H_5	Br

As can be seen from the structures disclosed in Table I, the α_2^{Br}, β_1^{Br}, γ_1^{Br}, α_2^{I}, β_1^{I}, γ_1^{I}, and δ_1^{I} components of the LL-E33288 antibiotic complex each contain a secondary amino group which is part of a substituted 4-aminopentose unit. It has now been discovered that the reaction of any of the above components with an unsubstituted or substituted, saturated or unsaturated alkyl or aryl acid anhydride, acid chloride or acyl cation equivalent results in the introduction of an acyl moiety on the secondary amino group as shown in Scheme I below.



Scheme I

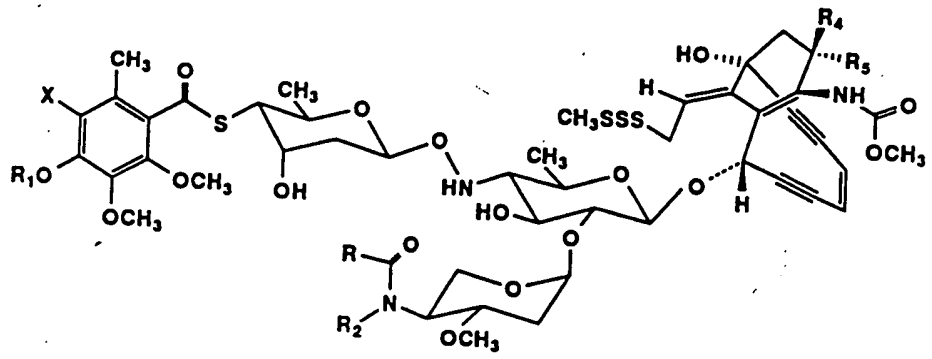
wherein W is the substituent attached to R_2NH- of the aminopentose in Table I, R is hydrogen or a branched or unbranched alkyl (C_1-C_{10}) or alkylene (C_1-C_{10}) group, an aryl or heteroaryl group, or an aryl-alkyl (C_1-C_5) or heteroaryl-alkyl (C_1-C_5) group, all optionally substituted by one or more hydroxy, amino, carboxy, halo, nitro, lower (C_1-C_3) alkoxy, or lower (C_1-C_5) thioalkoxy groups.

N-Acyl derivatives are also prepared from the dihydro derivatives of the LL-E33288 antibiotics, namely, dihydro-LL-E33288α_2^{Br}, dihydro-LL-E33288β_1^{Br}, dihydro-E33288γ_1^{Br}, dihydro-LL-E33288α_2^{I}, dihydro-LL-E33288β_1^{I}, dihydro-LL-E33288γ_1^{I}, and dihydro-LL-E33288δ_1^{I}, of parent application Serial No. 004,154.

As an example, reaction of LL-E33288γ_1^I with acetic anhydride in methanol produces N-acetyl-LL-E33288γ_1^I while the reaction of LL-E33288δ_1^I with the mixed anhydride of acetic acid and formic acid produces N-formyl-LL-E33288δ_1^I, both potent new antitumor antibiotics. The reaction of dihydro-LL-E33288γ_1^I with acetic anhydride in methanol produces N-acetyl-dihydro-LL-E33288γ_1^I. N-Acetyl-dihydro-LL-E33288γ_1^I is also produced by the reaction of N-acetyl-LL-E33288γ_1^I with sodium borohydride under the conditions described in Serial No. 004,154. Some of the chemical structures of the N-Acyl derivatives of the LL-E33288 and the dihydro-LL-E33288 anticancer antibiotics are shown in Table II below:

Table II

Proposed Structures for the N-Acyl Derivatives of the LL-E33288 and dihydro LL-E33288 Antibiotics



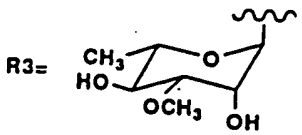


Table II (Cont'd)

Proposed Structures for the N-Acyl Derivatives of the LL-E33288 and dihydro LL-E33288 Antibiotics

Designation	R_1	R_2	R_4	R_5	X
N-Acyl-dihydro LL-E33288α_2^I	H	C_2H_5	OH	H	I
N-Acyl LL-E33288α_2^I	H	C_2H_5	=O		I
N-Acyl-dihydro LL-E33288β_1^I	R_3	$(CH_3)_2CH$	OH	H	I
N-Acyl LL-E33288β_1^I	R_3	$(CH_3)_2CH$	=O		I
N-Acyl-dihydro LL-E33288γ_1^I	R_3	C_2H_5	OH	H	I
N-Acyl LL-E33288γ_1^I	R_3	C_2H_5	=O		I
N-Acyl-dihydro LL-E33288δ_1^I	R_3	CH_3	OH	H	I
N-Acyl LL-E33288δ_1^I	R_3	CH_3	=O		I
N-Acyl-dihydro LL-E33288α_2^{Br}	H	C_2H_5	OH	H	Br
N-Acyl LL-E33288α_2^{Br}	H	C_2H_5	=O		Br
N-Acyl-dihydro LL-E33288β_1^{Br}	R_3	$(CH_3)_2CH$	OH	H	Br
N-Acyl LL-E33288β_1^{Br}	R_3	$(CH_3)_2CH$	=O		Br
N-Acyl-dihydro LL-E33288γ_1^{Br}	R_3	C_2H_5	OH	H	Br
N-Acyl LL-E33288γ_1^{Br}	R_3	C_2H_5	=O		Br

R = hydrogen or a branched or unbranched alkyl (C_1-C_{10}) or alkylene (C_1-C_{10}) group, an aryl or heteroaryl group, or an aryl-alkyl (C_1-C_5) or heteroaryl-alkyl (C_1-C_5) group, all optionally substituted by one or more hydroxy, amino, carboxy, halo, nitro, lower (C_1-C_3) alkoxy, or lower (C_1-C_5) thioalkoxy groups.

The physico-chemical characteristics of four of the N-acyl derivatives of the LL-E33288 antitumor antibiotics, namely, N-acetyl-LL-E33288δ_1^I, N-formyl-LL-E33288δ_1^I, N-acetyl-LL-E33288γ_1^I and N-acetyl-dihydro-LL-E33288γ_1^I are described below.

N-acetyl-LL-E33288δ_1^I

a) molecular weight: 1395, determined by FABMS;

b) molecular formula: $C_{56}H_{74}N_3O_{22}IS_4$, exact mass for M+K was determined by high resolution FABMS to be 1434.2329 for $C_{56}H_{74}N_3O_{22}IS_4K$; and

c) proton magnetic resonance spectrum: as shown in Figure I (300 MHz, $CDCl_3$).

N-formyl-LL-E33288δ_1^I

a) molecular weight: 1381, determined by FABMS;

b) molecular formula: $C_{55}H_{72}N_3O_{22}IS_4$, exact mass for M+K was determined by high resolution FABMS to be 1420.2172 for $C_{55}H_{72}N_3O_{22}IS_4K$; and

c) proton magnetic resonance spectrum: as shown in Figure II (300 MHz, $CDCl_3$).

N-acetyl-LL-E33288γ_1^I

a) molecular weight: 1409, determined by FABMS;

b) molecular formula: $C_{57}H_{76}N_3O_{22}IS_4$, exact mass for M+H was determined by high resolution FABMS to be 1410.2954 for $C_{57}H_{77}N_3O_{22}IS_4$;

c) Ultraviolet absorption spectrum: as shown in Figure III (methanol);

d) Infrared absorption spectrum: as shown in Figure IV (KBr disc);

N-acetyl-LL-E33288γ_1^I

e) Proton magnetic resonance spectrum: as shown in Figure V (300 MHz, $CDCl_3$);

f) Carbon-13 magnetic resonance spectrum: as shown in Figure VI (75.43 MHz, $CDCl_3$, ppm from TMS) significant peaks as listed below:

14.0 q	17.6 q	17.7 q	19.0 q	22.4 q	22.8 q
25.4 q	36.7 t	36.9 t	39.2 t	47.6 t	51.6 d
52.4 q	53.1 t	57.0 q	57.2 q	58.8 t	60.9 q
61.7 q	64.4 d	67.0 d	68.1 d	68.4 d	69.0 d
69.1 d	70.5 d	71.1 d	71.7 s	71.9 d	72.4 d
77.6 d	80.8 d	83.2 s	87.0 s	93.5 s	97.9 d
98.1 s	99.7 d	100.9 s	101.3 d	102.6 d	123.2 d
124.5 d	127.1 d	130.2 s	133.4 s	136.5 s	142.9 s
143.0 s	150.6 s	151.5 s	155.0 s	172.3 s	191.9 s
192.1 s					

N-acetyl-dihydro-LL-E33288γ_1^I

a) Ultraviolet absorption spectrum: as shown in Figure VII (methanol);

b) Proton magnetic resonance spectrum: as shown in Figure VIII (300 MHz, $CDCl_3$).

The N-acyl derivatives of the LL-E33288 antitumor antibiotics are most conveniently characterized by high-performance liquid chromatography (HPLC) and by thin-layer chromatography (TLC).

The preferred analytical HPLC system for the characterization of some of the N-acyl derivatives of the LL-E33288 antitumor antibiotics is shown below:

Column: Analytichem Sepralyte C_{18},5μ, 4.6 mm x 25 cm

Mobile Phase: 0.2M aqueous ammonium acetate, pH 6.0: acetonitrile, 50:50

Flow Rate: 1.5 ml/minute

Detection: UV_{254nm} and UV_{280nm}

Table III gives the approximate retention times of some of the N-acyl derivatives of the LL-E33288 antitumor antibiotics:

Table III

N-acyl-LL-E33288 Antitumor Antibiotics	Retention Time (minutes)
N-acetyl-LL-E33288γ_1^I	6.6
N-formyl-LL-E33288γ_1^I	6.2
N-acetyl-LL-E33288δ_1^I	4.5
N-formyl-LL-E33288δ_1^I	4.2
LL-E33288γ_1^I	8.0
LL-E33288δ_1^I	6.0

The preferred TLC system for the characterization of the N-acyl derivatives of the LL-E33288 antitumor antibiotics is shown below:

Adsorbent: Whatman High Performance TLC (HPTLC) plates, type LHP-KF;

Detection: Visualized by quenching effect under short wavelength UV lamp (254 nm);

Solvent System: Ethyl acetate saturated with 0.1M aqueous phosphate buffer at pH 7.0.

Table IV gives the approximate Rf values of some of the N-acyl derivatives of the LL-E33288 antitumor antibiotics in the TLC system above:

Table IV

N-acyl-LL-E33288 Antitumor Antibiotics	Rf
N-acetyl-LL-E33288γ_1^I	0.53
N-formyl-LL-E33288γ_1^I	0.53
N-acetyl-LL-E33288δ_1^I	0.25
N-formyl-LL-E33288δ_1^I	0.31
N-acetyl-dihydro-LL-E33288γ_1^I	0.38
N-monomethylsuccinyl-LL-E33288γ_1^I	0.42
LL-E33288γ_1^I	0.25
LL-E33288δ_1^I	0.14

The N-acyl derivatives of the LL-E33288 antitumor antibiotics are useful as antibacterial agents. The *in vitro* antibacterial activity of N-acetyl-LL-E33288δ_1^I, N-formyl-LL-E33288δ_1^I and N-acetyl-LL-E33288γ_1^I was determined against a spectrum of gram-positive and gram-negative bacteria by a standard agar dilution method. Mueller-Hinton agar containing two-fold decreasing concentrations of the antibiotics was poured into petri plates. The agar surfaces were inoculated with 1 to 5 x 10^4 colony forming units of bacteria by means of a Steers replicating device. The lowest concentration of N-acyl-LL-E33288 antitumor antibiotic that inhibited growth of a bacterial strain after about 18 hours of incubation at approximately 35°C was recorded as the minimal inhibitory concentration (MIC) for that strain. The results are summarized in Table V.

Table V

In vitro Antibacterial Activity of

N-Acyl-LL-E33288 Antibiotics

Organism		Minimal Inhibitory Concentration, mcg/ml		
		N-acetyl-LL-E33288δ_1^I	*N*-formyl-LL-E33288δ_1^I	*N*-acetyl-LL-E33288γ_1^I
Escherichia coli	CMC 84-11	2	2	>2
Escherichia coli	No. 311 (MP)	2	1	>2
Escherichia coli	ATCC 25922	1	1	>2
Klebsiella pneumoniae	CMC 84-5	8	4	>2
Klebsiella pneumoniae	AD (MP)	1	1	2
Enterobacter cloacae	CMC 84-4	4	4	>2
Serratia marcescens	F-35 (MP)	8	4	>2
Pseudomonas aeruginosa	12-4-4(MP)	4	2	>2
Pseudomonas aeruginosa	ATCC27853	4	2	>2
Staphylococcus aureus	Smith (MP)	0.12	0.06	0.008
Staphylococcus aureus	ATCC 25923	0.25	0.12	0.06
Staphylococcus epidermidis	ATCC 12228	0.015	0.03	0.12
Streptococcus faecalis	ATCC 29212	0.06	0.06	0.12
Streptococcus faecalis	IO 83-28	0.5	0.12	0.12

The N-acyl derivatives of the LL-E33288 antitumor antibiotics are also active as antitumor agents as determined in the Biochemical Induction Assay (BIA), a bacterial assay system which specifically measures the ability of an agent to directly or indirectly initiate DNA damage. The indicator organism for this test is an E. colilambda lysogen, genetically constructed such that a DNA damaging event results in the expression of the gene for the enzyme β-glactosidase. This enzyme can be determined qualitatively or quantitatively by a biochemical assay as an indication that DNA damage has occurred.

A modified version of the quantitative liquid BIA disclosed by Elespuru, R. and Yarmolinsky, M., Environmental Mutagenesis, 1, 65 (1979) was used to evaluate these compounds.

Certain in vivo testing systems and protocols have been developed by the National Cancer Institute for testing compounds to determine their suitability as anti-neoplastic agents. These have been reported in "Cancer Chemotherapy Reports", Part III, Vol. 3, No. 2 (1972), Geran, et. al. These protocols have established standardized screening tests which are generally followed in the field of testing for anti-tumor agents. Of these systems, lymphocytic leukemia P388, melanotic melanoma B16 and colon 26 adenocarcinoma are particularly significant to the present invention. These neoplasms are utilized for testing as transplantable tumors in mice. Generally, significant anti-tumor activity, shown in these protocols by a percentage increase of mean survival times of the treated animals (T) over the control animals (C), is indicative of similar results against human leukemias and solid tumors.

Lymphocytic Leukemia P388 Test

The animals used were BDF_1 mice, all of one sex, weighing a minimum of 17 g and all within a 3 g weight range. There were 5 or 6 mice per test group. The tumor transplant was by intraperitoneal injection of 0.5 ml of dilute ascitic fluid containing 10^6 cells of lymphocytic leukemia P388. The test compounds were administered intraperitoneally in a volume of 0.5 ml of 0.2% Klucel in normal saline on days 1, 5 and 9 (relative to tumor inoculation) at the indicated doses. The mice were weighed and the survivors recorded on a regular basis for 30 days. The median survival time and the ratio of survival time for treated (T)/control (C) animals were calculated. The parent antitumor antibiotic, LL-E33288γ_1^I, was used as positive control.

The test results of N-acetyl-LL-E33288δ_1^I, N-formyl-LL-E33288δ_1^I and N-acetyl-LL-E33288γ_1^I are summarized in Table VI. If T/C x 100 (%) is 125 or over, the tested compound is considered to have significant anti-tumor activity.

Table VI

Lymphocytic Leukemia P388 Test

Compound	Dose (mg/Kg)	Median survival (days)	T/C X 100 (%)
saline		11.0	
N-acetyl-LL-E33288δ_1^I	0.1	13.0	118
	0.05	29.5	268
	0.025	26.0	236
	0.0125	20.0	182
	0.006	20.0	182
N-acetyl-LL-E33288δ_1^I	0.1	11.5	105
	0.05	30.0	273
	0.025	25.0	227
	0.0125	23.0	209
	0.006	19.5	177
N-formyl-LL-E33288δ_1^I	0.1	12.5	114
	0.05	27.0	245
	0.025	22.5	205
	0.0125	21.0	191
	0.006	20.5	186
LL-E33288γ_1^I	0.01	13.0	118
	0.005	25.0	227
	0.0025	30.0	273
	0.00125	26.5	241

Table VI (Cont'd)
Lymphocytic Leukemia P388 Test

saline			11.0	
N-acetyl-LL-E33288γ_1I		0..08	18	164
		0.04	29.5	268
		0.02	28.0	255
		0.005	17.5	159
		0.0025	14.0	127
		0.00125	13.5	123
LL-E33288γ_1I		0.01	22.5	205
		0.005	26.0	236
		0.0025	24.5	223
		0.00125	21.0	191
		0.0006	19.0	173

Melanotic Melanoma B16 Test

The animals used were BDF_1 mice, all of the same sex, weighing a minimum of 17 g and all within a 3 g weight range. There are normally 6 animals per test group. A 1 g portion of melanoma B16 tumor was homogenized in 10 ml of cold balanched salt solution and a 0.5 ml aliquot of the homogenate was implanted intraperitoneally into each of the test mice. The test compounds were administered intraperitoneally on days 1 through 9 (relative to tumor inoculation) at various doses. The mice were weighed and survivors recorded on

a regular basis for 60 days. The median survival time and the ratio of survival time for treated (T)/control (C) animals was calculated. The parent antitumor antibiotic LL-E33288γ_1^I was used as positive control.

The test results of N-acetyl-LL-E33288δ_1^I and N-acetyl-LL-E33288γ_1^I are summarized in Table VII. If T/C x 100 (%) is 125 or over, the tested compound is considered to have significant anti-tumor activity.

Table VII

Melanotic Melanoma B16 Test

Compound	Dose (mg/Kg)	Median survival (days)	T/C X 100 (%)
saline		21.0	
N-acetyl-LL-E33288δ_1^I	0.025	35.5	169
	0.0125	27.5	131
	0.006	26.0	124
	0.003	25.0	119
	0.0015	21.5	102
LL-E33288γ_1^I	0.0025	39.0	186
	0.00125	39.0	186
	0.0006	35.0	167
	0.0003	29.5	140
	0.00015	24.5	117
saline		21.0	
N-acetyl-LL-E33288γ_1^I	0.025	26.0	124
	0.0125	38.0	181
	0.006	39.0	186
	0.003	33.5	160
	0.0015	26.5	126
	0.0007	26.0	124
	0.00035	24.5	116
	0.00017	23.5	112

Table VII (Cont'd)
Melanotic Melanoma B16 Test

LL-E33288γ_1^I	0.005	8.0	38
	0.0025	27.0	129
	0.00125	41.5	198
	0.0006	45.0	214
	0.0003	35.5	169
	0.00015	35.0	167
	0.00007	34.5	164
	0.00003	31	148

Colon 26 Adenocarcinoma Test

The animals used were CD_2F_1 female mice weighing a minimum of 17 g and all within a 3 g weight range. There were 5 or 6 mice per test group with three groups of 5 or 6 animals used as untreated controls for each test. The tumor implant was by intraperitoneal injection of 0.5 ml of a 2% colon 26 tumor brei in Eagle's MEM medium containing antibacterial agent. The test compounds were administered intraperitoneally on days 1, 5 and 9 (relative to tumor implant doses). The mice were weighed and deaths recorded on a regular basis for 30 days. The median survival times for treated (T)/control (C) animals were calculated. The parent antitumor antibiotic LL-E33288γ_1^I was used as positive control.

The test results of N-acetyl-LL-E33288δ_1^I are summarized in Table VIII. If T/C x 100 (%) is 130 or over, the tested compound is considered to have significant antitumor activity.

Table VIII

Colon 26 Adenocarcinoma Test

Compound	Dose (mg/Kg)	Median survival (days)	T/C X 100 (%)
saline		16.0	
N-acetyl-LL-E33288δ_1^I	0.05	22.5	141
	0.025	40.0	250
	0.0125	21.0	131
	0.006	24.5	153
	0.003	19.0	119
	0.0015	19.0	119
	0.0007	19.0	119
LL-E33288γ_1^I	0.01	14.0	88
	0.005	35.0	219
	0.0025	21.5	134
	0.00125	24.0	150
	0.0006	19.5	122
	0.0003	18.0	113
	0.00015	17.5	109

The invention is further described by the following non-limiting examples.

Example 1

Preparation and purification of N-acetyl-LL-E33288δ_1^I

Acetic anhydride (2 ml) was added dropwise to a stirred methanolic solution of partially purified LL-E33288δ_1^I (608 mg, 57% pure, in 60 ml) cooled in an ice-water bath. The reaction mixture was allowed to stir at 0°C for 1 hour, then warmed slowly to room temperature and the reaction was allowed to continue for another 3 hours. The reaction mixture was then concentrated in vacuo and the residue was taken up in a mixture of 60 ml each of dichloromethane and water. The aqueous phase was neutralized with dilute aqueous sodium hydroxide in order to remove as much of the acetic acid from the organic phase as possible. The organic phase was separated, dried over anhydrous sodium sulfate, concentrated to a small volume and was precipitated by addition of hexanes to give 604 mg of crude N-acetyl-LL-E33288δ_1^I.

The crude N-acetyl-LL-E33288δ_1^I above was dissolved in 8 ml of acetonitrile:0.2M ammonium acetate, pH 6.0 (35:65) and was chromatographed in four batches on a Sepralyte C_{18} column (1.5 x 21 cm). The columns were eluted at 10 ml/min. first with acetonitrile:0.2M ammonium acetate pH 6.0 (35:65) for 30 minutes followed by a linear gradient to acetonitrile:0.2M ammonium acetate, pH 6.0 (40:60) over 60 minutes. Throughout the chromatography the column eluents were monitored at $UV_{254\ nm}$ and fractions were collected every 2.5 minutes. Peak fractions were analyzed by HPLC and those containing pure N-acetyl-LL-E33288δ_1^I according to the HPLC analysis were pooled and concentrated in vacuo to remove acetonitrile. The N-acetyl-LL-E33288δ_1^I present in the aqueous mixture was extracted into ethyl acetate and the ethyl acetate phase was dried over anhydrous sodium sulfate, concentrated to a small volume and was precipitated by addition of

hexanes to give 161 mg of semi-purified N-acetyl-LL-E33288δ_1^I.

TLC analysis (E. Merck Silica gel 60 F_{254} precoated aluminum sheets, 0.2 mm, 3% isopropanol in ethyl acetate saturated with 0.1M potassium dihydrogen phosphate, detected by bioautography using the agar biochemical induction assay) showed that the semi-purified N-acetyl-LL-E33288δ_1^I sample from above contained trace amounts of unreacted LL-E33288δ_1^I.

The semi-purified N-acetyl-LL-E33288δ_1^I (160 mg) was dissolved in 1 ml of ethyl acetate and chromatographed on a Bio-Sil A (20-44 μ, Bio-Rad Laboratories) column (1.5 cm x 90 cm) packed and equilibrated with ethyl acetate. The column was first eluted with ethyl acetate at a flow rate of 3.6 ml/minute for 3.5 hours, collecting 18 ml fractions. The eluent was changed to 3% isopropanol in ethyl acetate saturated with 0.1M potassium dihydrogen phosphate and elution continued for another 3.5 hours. The fractions were analyzed by TLC as before and those contain pure N-acetyl-LL-E33288δ_1^I (fractions 58-64) were pooled, concentrated in vacuo to dryness, redissolved in a small amount of ethyl acetate and was precipitated by addition of hexanes to give 118 mg of analytically pure N-acetyl-LL-E33288δ_1^I, containing no detectable amounts of the un-acylated parent antitumor antibiotic. The proton magnetic resonance spectrum is shown in Figure I.

Example 2

Preparation and purification of N-formyl-LL-E33288δ_1^I

The mixed anhydride of acetic acid and formic acid was freshly prepared by addition of 200 μl of formic acid dropwise to 400 μl of acetic anhydride cooled in an ice water bath. The reaction mixture was then warmed at 50°C for 5 minutes to complete the anhydride exchange and was then kept at 0°C. The mixed anhydride of acetic acid and formic acid (100 μl) prepared above

was added dropwise to a stirred methanolic solution of partially purified LL-E33288δ_1^I (92 mg, 45% pure, in 30 ml) cooled in an ice-water bath. The reaction mixture was allowed to stir at 0°C for 45 minutes, hexanes (20 ml) was then added to the reaction mixture and the mixture was concentrated in vacuo to mean dryness. The residue was redissolved in ethyl acetate and precipitated by addition of hexanes to give a chunky, sticky precipitate which was collected by centrifugation. The precipitate was redissolved in a small amount of ethyl acetate and precipitated again by addition of hexanes to give crude N-formyl-LL-E33288δ_1^I.

The crude N-formyl-LL-E33288δ_1^I sample from above was partially purified by preparative TLC on silica gel (two of Analtech Silica Gel GF precoated plates, 2,000 μ, 20 x 20 cm) eluting with ethyl acetate saturated with phosphate buffer at pH 7.0. The desired band was excised and the N-formyl-LL-E33288δ_1^I was recovered by washing the silica gel with methylene chloride:methanol (80:20) to give, upon workup, 110 mg of partially purified N-formyl-LL-E33288δ_1^I.

The partially purified N-formyl-LL-E33288δ_1^I from above was dissolved in 1 ml of acetonitrile:ammonium acetate, pH 6.0 (35:65) and was chromatographed on a Sepralyte C_{18} column (1.5 x 20 cm). The column was eluted at 8 ml/minute with acetonitrile:0.2M ammonium acetate, pH 6.0 (35:65) for 1.75 hours, monitoring at UV_{254nm} and collecting 20 ml fractions. Peak fractions were analyzed by HPLC and those containing pure N-formyl-LL-E33288δ_1^I according to the HPLC analysis were pooled and concentrated in vacuo to remove acetonitrile. The cloudy aqueous mixture, containing N-formyl-LL-E33288δ_1^I was extracted with ethyl acetate and the ethyl acetate phase was concentrated to dryness. The residue was redissolved in methylene chloride, dried over anhydrous sodium sulfate, concentrated

and precipitated by addition of hexanes to give 36.5 mg of semi-purified N-formyl-LL-E33288δ_1^I.

TLC analysis (E. Merck Silica gel 60 F254 precoated aluminium sheets, 0.2 mm, 3% isopropanol in ethyl acetate saturated with 0.1M potassium hydrogen phosphate, detected by bioautography using the agar biochemical induction assay) showed that the semi-purified N-formyl-LL-E33288δ_1^I sample above contained trace amounts of unreacted LL-E33288δ_1^I and γ_1^I. The semi-purified N-formyl-LL-E33288δ_1^I (36.5 mg) was dissolved in 1 ml of ethyl acetate and chromatographed on a Bio-Sil A (20-44 μ, Bio-Rad Laboratories) column (1.5 cm x 23 cm) packed and equilibrated with ethyl acetate. The column was first eluted with ethyl acetate at a flow rate of 1.2 ml/minute for 2 hours, collecting 6 ml fractions. The eluent was changed to ethyl acetate:methanol (97:3) and elution continued for another 2 hours. The fractions were analyzed by TLC (E. Merck Silica gel 60 F254 precoated aluminium sheets, 0.2 mm, 3% isopropanol in ethyl acetate saturated with 0.1M potassium hydrogen phosphate, detected by spraying with a solution of 3% cupric acetate in 8% aqueous phosphoric acid) and those contained pure N-formyl-LL-E33288δ_1^I (fractions 35-38) were pooled, concentrated <u>in vacuo</u> to dryness. The residue was redissolved in a small amount of ethyl acetate, and precipitated by addition of hexanes to give an N-acetyl-LL-E33288δ_1^I sample which was still contaminated with trace amount of unreacted LL-E33288γ_1^I. This sample was chromatographed again on a Bio-Sil A column (0.8 x 20 cm) packed and equilibrated with ethyl acetate. The column was eluted with ethyl acetate at a flow rate of 1.2 ml/minute for 4 hours, collecting 6 ml fractions. The fractions were analyzed by TLC as before and those contained pure N-formyl-LL-E33288δ_1^I (fractions 14-33) were pooled and worked up as before to give 12.2 mg of analytically pure N-formyl-LL-E33288δ_1^I, containing no

detectable amounts of the un-acylated parent antibiotic. The proton magnetic resonance spectrum is displayed in Figure II.

Example 3

Preparation and purification of N-formyl-LL-E33288δ_1^I

The mixed anhydride of acetic acid and formic acid (750 μl) freshly prepared as described in Example 2 was added dropwise to a stirred methanolic solution of partially purified LL-E33288δ_1^I (689 mg, 70% pure, in 150 ml) cooled in an ice-water bath. The reaction mixture was allowed to stir at 0°C for one hour, excess hexanes was then added to the reaction mixture and the mixture was concentrated in vacuo to about 75 ml. Ethyl acetate (about 200 ml) was added to the solution and the mixture was concentrated to about 50 ml and crude N-formyl-LL-E33288δ_1^I (676 mg) was precipitated by addition of 300 ml of hexanes.

The crude N-formyl-LL-E33288δ_1^I was dissolved in 3 ml of ethyl acetate and chromatographed on a Bio-Sil A (40-80 μ) column (2.5 x 95 cm) packed and equilibrated in ethyl acetate. The column was eluted at 10 ml/min with ethyl acetate until the yellow band was off the column (1.75 hours). It was then eluted at 5 ml/min with ethyl acetate saturated with 0.1M potassium dihydrogen phosphate for another 5 hours. Throughout the chromatography 20 ml fractions were collected. The fractions were analyzed by TLC (E. Merck Silica gel 60 F254 precoated aluminium sheets, 0.2 mm, 3% isopropanol in ethyl acetate saturated with 0.1M potassium dihydrogen phosphate, detected by spraying with a solution of 3% cupric acetate in 8% aqueous phosphoric acid)and the major N-formyl-LL-E33288δ_1^I containing fractions (92-98) were pooled and worked up by concentration and precipitation to give 294 mg of partially purified N-formyl-LL-E33288δ_1^I. TLC analysis (detected by bioautography using the agar biochemical

induction assay) of this sample showed it to be free of any unreacted LL-E33288δ_1^I.

The partially purified N-formyl-LL-E33288δ_1^I was dissolved in 4 ml of acetonitrile:0.2M ammonium acetate, pH 6.0 (35:65) and was chromatographed in two batches on a Sepralyte C_{18} column (1.5 x 45 cm) equilibrated with acetonitrile:0.2M ammonium acetate, pH 6.0 (35:65). The column was eluted at 8 ml/min with the same solvent for 3 hours, monitoring at UV_{254nm} and collecting 20 ml fractions. Peak fractions were analyzed by HPLC and those containing pure N-formyl-LL-E33288δ_1^I according to the HPLC analysis were pooled and concentrated <u>in vacuo</u> to remove acetonitrile. The N-formyl-LL-E33288δ_1^I present in the aqueous mixture was extracted into ethyl acetate and worked up by concentration and precipitation to give 161 mg of pure N-formyl-LL-E33288δ_1^I. The proton magnetic resonance spectrum is displayed in Figure II.

Example 4

Preparation of N-acetyl-LL-E33288γ_1^I

Acetic anhydride (4 ml) was added dropwise to a stirred methanolic solution of partially purified LL-E33288γ_1^I (1.25 g, 85% pure, in 100 ml of methanol) cooled in an ice-water bath. The reaction mixture was allowed to stir at 0°C for 1 hour, then warmed slowly to room temperature and the reaction was allowed to continue for another 2 hours. The reaction mixture was then concentrated <u>in vacuo</u> and the residue was taken up in a mixture of 100 ml each of dichloromethane and water. The aqueous phase was neutralized with dilute aqueous sodium hydroxide in order to remove most of the acetic acid from the organic phase. The organic phase was separated, dried over anhydrous sodium sulfate, concentrated to a small volume and the product was precipitated by addition of hexanes to give 1.18 g of 80% pure N-acetyl-LL-E33288γ_1^I which can be purified following procedures described in Example 1 to give pure

N-acetyl-LL-E33288γ_1^I. The ultraviolet, infrared, proton and carbon-13 spectrums are displayed in Figures III-VI.

Example 5

Preparation of N-formyl-LL-E33288γ_1^I

The mixed anhydride of acetic acid and formic acid (100 µl) freshly prepared as described in Example 2 was added dropwise to a stirred methanolic solution of analytically pure LL-E33288γ_1^I (49.6 mg, in 50 ml of methanol) cooled in an ice-water bath. The reaction mixture was allowed to stir at 0°C for one hour followed by at room temperature overnight. It was then concentrated to dryness, redissolved in a small volume of ethyl acetate and the product was precipitated by addition of hexane. The dried precipitate was redissolved in 10 ml of methanol and treated again with the mixed anhydride of acetic acid and formic acid (400 µl). The reaction mixture was allowed to stir at room temperature for 2 hours and was worked up by concentration and precipitation as described before to give crude N-formyl-LL-E33288γ_1^I as an off-white solid. The crude N-formyl-LL-E33288γ_1^I was purified by preparative TLC (two 20 cm x 20 cm Analtech tapered Silica Gel GF plates, eluted with 3% isopropanol in ethyl acetate saturated with 0.1M potassium dihydrogen phosphate)to give semi-purified N-formyl-LL-E33288γ_1^I.

Example 6

Preparation of N-acetyl-dihydro-LL-E33288γ_1^I

A 2 ml portion of methyl iodide was added to a solution of 25 mg of N-acetyl-LL-E33288γ_1^I (prepared as described in Example 4) in 8 ml of absolute ethanol and the mixture was cooled in an ice-water bath. To this was added one ml of a 0.4M ethanolic solution of sodium borohydride in two equal portions. When the reaction was complete (10 minutes after addition of the second portion of sodium borohydride solution), the borate complex was decomposed by the addition of 400 µl

of a 4M ethanolic solution of acetic acid. The reaction mixture was then concentrated to a golden yellow residue which was redissolved in 10 ml of ethyl acetate, diluted with 10 ml of dichloromethane and re-concentrated to dryness. This residue was redissolved in ethyl acetate, the insoluble borate salt was filtered off, and the solution was concentrated to dryness to give an off-white solid which was suspended in 4 ml of water and passed through a Bond Elut™ (Analytichem International) C_{18} cartridge. The cartridge was sequentially eluted with 4 ml each of water, methanol:water (1:1) and methanol. The methanol eluate, containing most of the N-acetyl-dihydro-LL-E33288γ_1^I, was concentrated to give an off-white solid and was further purified by preparative TLC (Analtech Silica Gel GF, 20 x 20 cm, 1000 μ layer thickness, ethyl acetate:methanol, 97:3 elution) to give analytically pure N-acetyl-dihydro-LL-E33288γ_1^I. The ultraviolet and proton magnetic resonance spectrum is displayed in Figure VII and VIII.

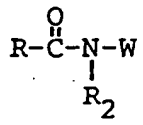
Example 7

Preparation of N-monomethylsuccinyl-LL-E33288γ_1^I

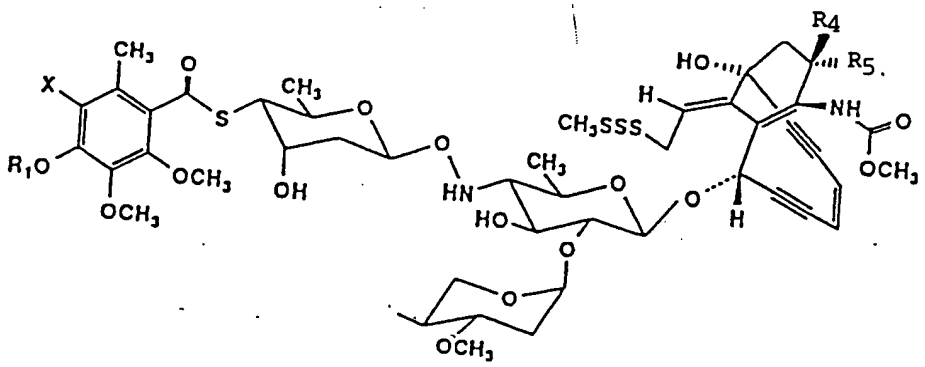
The anhydride of the monomethyl ester of succinic acid (55 mg) was added in three portions to a solution of LL-E33288γ_1^I (12.3 mg) in methanol (2 ml) and kept at room temperature for a three day period. The reaction mixture was concentrated to dryness and the residue was redissolved in a small volume of ethyl acetate and precipitated by addition of hexane. The gummy precipitate was triturated thoroughly with diethyl ether and was then redissolved in a small volume of ethyl acetate and precipitated by the addition of diethyl ether and hexane to give crude N-monomethylsuccinyl-LL-E33288γ_1^I.

CLAIMS

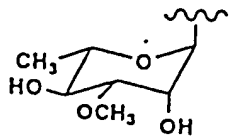
1. A compound of the formula



wherein W is

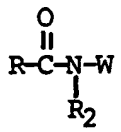


R is hydrogen or a branched or unbranched alkyl (C_1-C_{10}) or alkylene (C_1-C_{10}) group, an aryl or hetero-aryl group, or an aryl-alkyl (C_1-C_5) or hetero-aryl-alkyl (C_1-C_5) group, all optionally substituted by one or more hydroxy, amino, carboxy, halo, nitro, lower (C_1-C_3) alkoxy, or lower (C_1-C_5) thioalkoxy groups; R_1 is H or

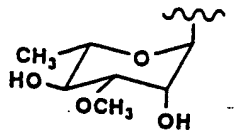


R_2 is CH_3, C_2H_5 or $CH(CH_3)_2$; R_4 is OH when R_5 is H or R_4 and R_5 taken together are a carbonyl; and X is an iodine or bromine atom.

2. A compound according to Claim 1 of the formula:



which is the antitumor antibiotic N-acetyl-LL-E33288δ_1^I, wherein W is hereinbefore defined; R is CH_3; R_1 is



R_2 is CH_3; R_4 and R_5 taken together is a carbonyl; X is iodine and having:

a) a proton magnetic resonance spectrum as shown in Figure I;

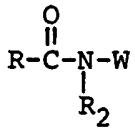
b) a molecular weight of 1395 as determined by FABMS;

c) a molecular formula of $C_{56}H_{74}N_3O_{22}IS_4$ with an exact mass for M+K as determined by high resolution FAB-MS to be 1434.2329 for $C_{56}H_{74}N_3O_{22}IS_4K$;

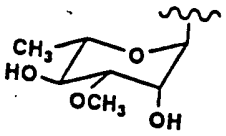
d) a retention time of 4.5 minutes by HPLC using a Analytichem Sepralyte C_{18}, 5µ, 4.6 mm x 25 cm column with a mobile phase of 0.2M aqueous ammonium acetate at pH 6.0, made 1:1 with acetonitrile and having a flow rate of 1.5 ml/minute with UV detection at 254 nm and 280 nm; and

e) a Rf of 0.25 on Whatman High Performance TLC (HPTLC) plates, type LHP-KF using ethyl acetate saturated with 0.1M aqueous phosphate buffer at pH 7.0, visualized using a 254 nm UV lamp.

3. A compound according to Claim 1 of the formula:



which is the antitumor antibiotic N-formyl-LL-E33288δ_1^I, wherein W is hereinbefore defined; R is H; R_1 is



R_2 is CH_3; R_4 and R_5 taken together is a carbonyl; X is iodine and having:

a) a protonmagnetic resonance spectrum as shown in Figure II;

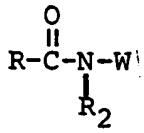
b) a molecular weight of 1381 as determined by FAB-MS;

c) a molecular formula of $C_{55}H_{72}N_3O_{22}IS_4$ with an exact mass for M+K as determined by high resolution FAB-MS to be 1420.2172 for $C_{55}H_{72}N_3O_{22}IS_4K$;

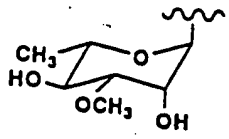
d) a retention time of 4.2 minutes by HPLC using an Analytichem Sepralyte C_{18}, 5μ, 4.6 mm x 25 cm column with a mobile phase of 0.2M aqueous ammonium acetate at pH 6.0, made 1:1 with acetonitrile and having a flow rate of 1.5 ml/minute with UV detection at 254 nm and 280 nm; and

e) a Rf of 0.31 on Whatman High Performance TLC (HPTLC) plates, Type LHP-KF using ethyl acetate saturated with 0.1M aqueous phosphate buffer at pH 7.0, visualized using a 254 nm UV lamp.

4. A compound according to Claim 1 of the formula:



which is the antitumor antibiotic N-acetyl-LL-E33288γ_1^I, wherein W is hereinbefore defined; R is CH_3; R_1 is



R_2 is C_2H_5; R_4 and R_5 taken together is a carbonyl; X is iodine and having:

a) a ultraviolet spectrum as shown in Figure III;

b) an infrared absorption spectrum as shown in Figure IV;

c) a proton magnetic resonance spectrum as shown in Figure V; and

d) a carbon-13 magnetic resonance spectrum as shown in Figure VI with significant peak listed as:

14.0 q	17.6 q	17.7 q	19.0 q	22.4 q	22.8 q
25.4 q	36.7 t	36.9 t	39.2 t	47.6 t	51.6 d
52.4 q	53.1 t	57.0 q	57.2 q	58.8 t	60.9 q
61.7 q	64.4 d	67.0 d	68.1 d	68.4 d	69.0 d
69.1 d	70.5 d	71.1 d	71.7 s	71.9 d	72.4 d
77.6 d	80.8 d	83.2 s	87.0 s	93.5 s	97.9 d
98.1 s	99.7 d	100.9 s	101.3 d	102.6 d	123.2 d
124.5 d	127.1 d	130.2 s	133.4 s	136.5 s	142.9 s
143.0 s	150.6 s	151.5 s	155.0 s	172.3 s	191.9 s
192.1 s					

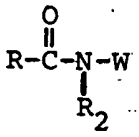
e) a molecular weight of 1409 as determined by FAB-MS;

f) a molecular formula of $C_{57}H_{76}N_3O_{22}IS_4$ with an exact mass for M+H as determined by high resolution FAB-MS to be 1410.2954 for $C_{57}H_{76}N_3O_{22}IS_4$;

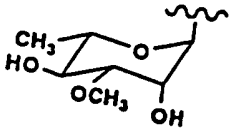
g) a retention time of 6.6 minutes by HPLC using an Analytichem Sepralyte C_{18}, 5µ, 4.6 mm x 25 cm column with a mobile phase of 0.2M aqueous ammonium acetate at pH 6.0, made 1:1 with acetonitrile and having a flow rate of 1.5 ml/minute with UV detection at 254 mm and 280 nm; and

h) a Rf of 0.53 on Whatman High Performance TLC (HPTLC) plates, type LHP-KF using ethyl acetate saturated with 0.1M aqueous phosphate buffer at pH 7.0, visualized using a 254 nm UV lamp.

5. A compound according to Claim 1 of the formula:



which is the antitumor antibiotic N-acetyl-dihydro-LL-E33288γ_1^I, wherein W is hereinbefore defined; R is CH_3; R_1 is



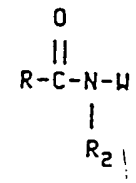
R_2 is C_2H_5; R_4 is OH; R_5 is H; X is iodine; and having

a) a ultraviolet absorption spectrum as shown in Figure VII;

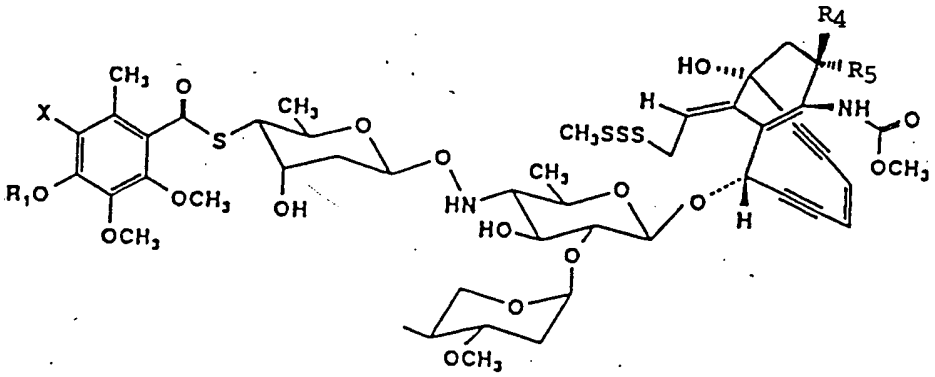
b) a proton magnetic resonance spectrum as shown in Figure VIII, and

c) a Rf of 0.38 on Whatman High Performance TLC (HPTLC) plates, type LHP-KF using ethyl acetate saturated with 0.1M aqueous phosphate buffer at pH 7.0, visualized using a 254 nm UV lamp.

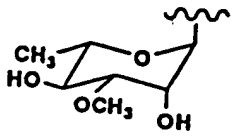
6. A process for producing an N-acyl derivative of the formula:



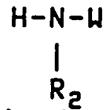
wherein W is



R is hydrogen or a branched or unbranched alkyl (C_1-C_{10}) or alkylene (C_1-C_{10}) group, an aryl or heteroaryl group, or an aryl-alkyl (C_1-C_5) or heteroaryl-alkyl (C_1-C_5) group, all optionally substituted by one or more hydroxy, amino, carboxy, halo, nitro, lower (C_1-C_3) alkoxy, or lower (C_1-C_5) thioalkoxy groups; R_1 is H or

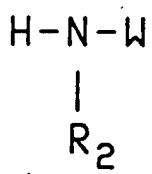


R_2 is CH_3, C_2H_5 or $CH(CH_3)_2$; R_4 is OH when R_5 is H or R_4 and R_5 taken together are a carbonyl; and X is an iodine or bromine atom prepared from a compound of the formula:



and designated as the antibiotic LL-E33288, α_2^{Br}, β_1^{Br}, γ_1^{Br}, α_2^{I}, β_1^{I}, γ_1^{I}, δ_1^{I}, and their dihydro counterparts which comprises

reacting the antibiotic



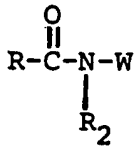
with an appropriately substituted anhydride, acid chloride, the mixed anhydride of acetic and formic acids or the anhydride of the monomethyl ester of succinic acid in methyl alcohol at a temperature of between -5°C to about +5°C for a period of one hour and at ambient temperature for one to twenty four hours,

precipitating from ethyl acetate with hexanes,

purifying by chromatography,

or to prepare the dihydro counterparts

reacting the N-acyl derivative of the formula:

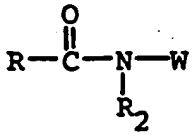


from those of the above in a methyl iodide, alcohol solution at a temperature of between $-5^{o}C$ to about $+5^{o}C$, with an alcoholic solution of sodium borohydride from 5 minutes to 5 hours,

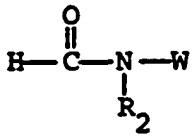
decomposing the borate complex with ethanolic acetic acid and

purifying the desired dihydro product by chromatography.

7. A process according to Claim 6 for producing a compound of the formula:

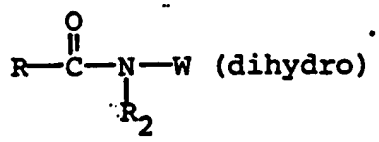


where R is CH_3 or H; R_2 is CH_3, CH_3CH_2 or $(CH_3)_2CH$, by reacting

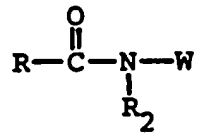


where R_2 is CH_3, CH_3CH_2 or $(CH_3)_2CH$, with acetic anhydride or the mixed anhydride of acetic and formic acids in methanol at -5° to $+5^{\circ}C$ for about one hour.

8. A process according to Claim 6 for producing a compound of the formula:

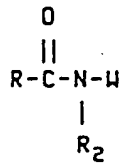


where R is CH_3 or H; R_2 is CH_3, CH_3CH_2 or $(CH_3)_2CH$, by reacting

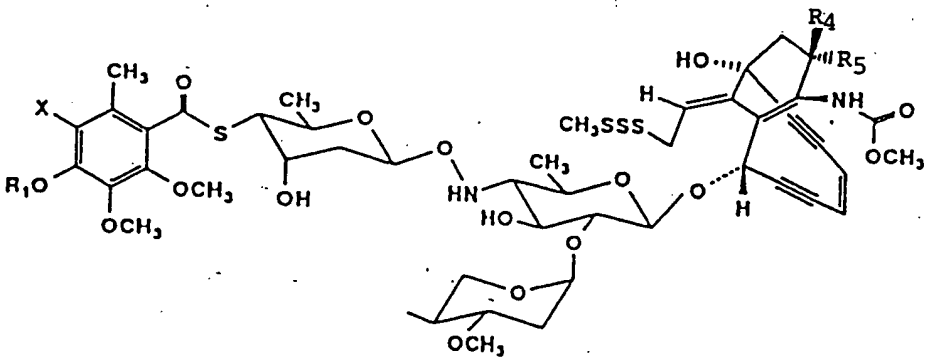


where R_2 is CH_3, CH_3CH_2 or $(CH_3)_2CH$, with sodium borohydride in an alcoholic solution at $-5^{\circ}C$ to about $+5^{\circ}C$ from 5 minutes to 5 hours.

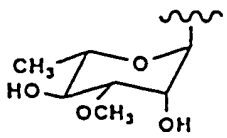
9. A substance or composition for use in a method of treating bacterial infections in warm-blooded animals, said substance or composition comprising a compound of the formula:



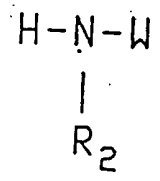
wherein W is



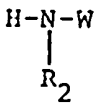
R is hydrogen or a branched or unbranched alkyl (C_1-C_{10}) or alkylene (C_1-C_{10}) group, an aryl or hetero-aryl group, or an aryl-alkyl (C_1-C_5) or hetero-aryl-alkyl (C_1-C_5) group, all optionally substituted by one or more hydroxy, amino, carboxy, halo, nitro, lower (C_1-C_3) alkoxy, or lower (C_1-C_5) thioalkoxy groups; R_1 is H or



R_2 is CH_3, C_2H_5 or $CH(CH_3)_2$; R_4 is OH when R_5 is H or R_4 and R_5 taken together are a carbonyl; and X is an iodine or bromine atom which are prepared from a compound of the formula:

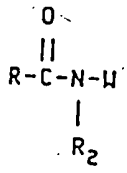


wherein

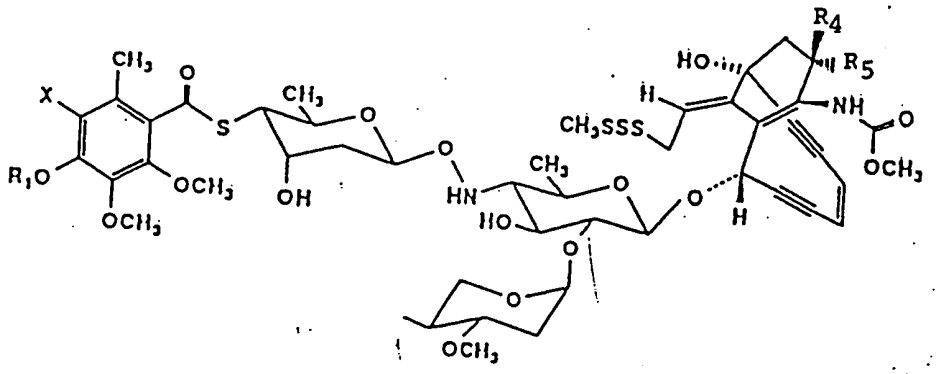


is designated as LL-E33288, α_2^{Br}, β_1^{Br}, γ_1^{Br}, α_2^{I}, β_1^{I}, γ_1^{I}, δ_1^{I}, and their dihydro counterparts, and said method comprising administering to said animals an antibacterially effective amount of said substance or composition.

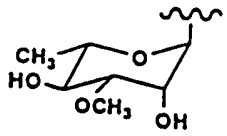
10. A method of inhibiting the growth of tumors in warm-blooded animals, said method comprising a compound of the formula:



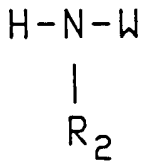
wherein W is



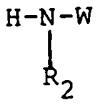
R is hydrogen or a branched or unbranched alkyl (C_1-C_{10}) or alkylene (C_1-C_{10}) group, an aryl or heteroaryl group, or an aryl-alkyl (C_1-C_5) or heteroaryl-alkyl (C_1-C_5) group, all optionally substituted by one or more hydroxy, amino, carboxy, halo, nitro, lower (C_1-C_3) alkoxy, or lower (C_1-C_5) thioalkoxy groups; R_1 is H or



R_2 is CH_3, C_2H_5 or $CH(CH_3)_2$; R_4 is OH when R_5 is H or R_4 and R_5 taken together are a carbonyl; and X is an iodine or bromine atom which are prepared from a compound of the formula:



wherein



is designated as LL-E33288, α_2^{Br}, β_1^{Br}, γ_1^{Br}, α_2^{I}, β_1^{I}, γ_1^{I}, δ_1^{I}, and their dihydro counterparts, and said method comprising administering to said animals an oncolytic amount of said substance or composition.

11. A substance or composition for a new use in a method of treatment, substantially as herein described and illustrated.

12. A process for producing an N-acyl derivative having the formula defined in Claim 6, substantially as herein described and illustrated.

DATED THIS 12 DAY OF DECEMBER 1986.

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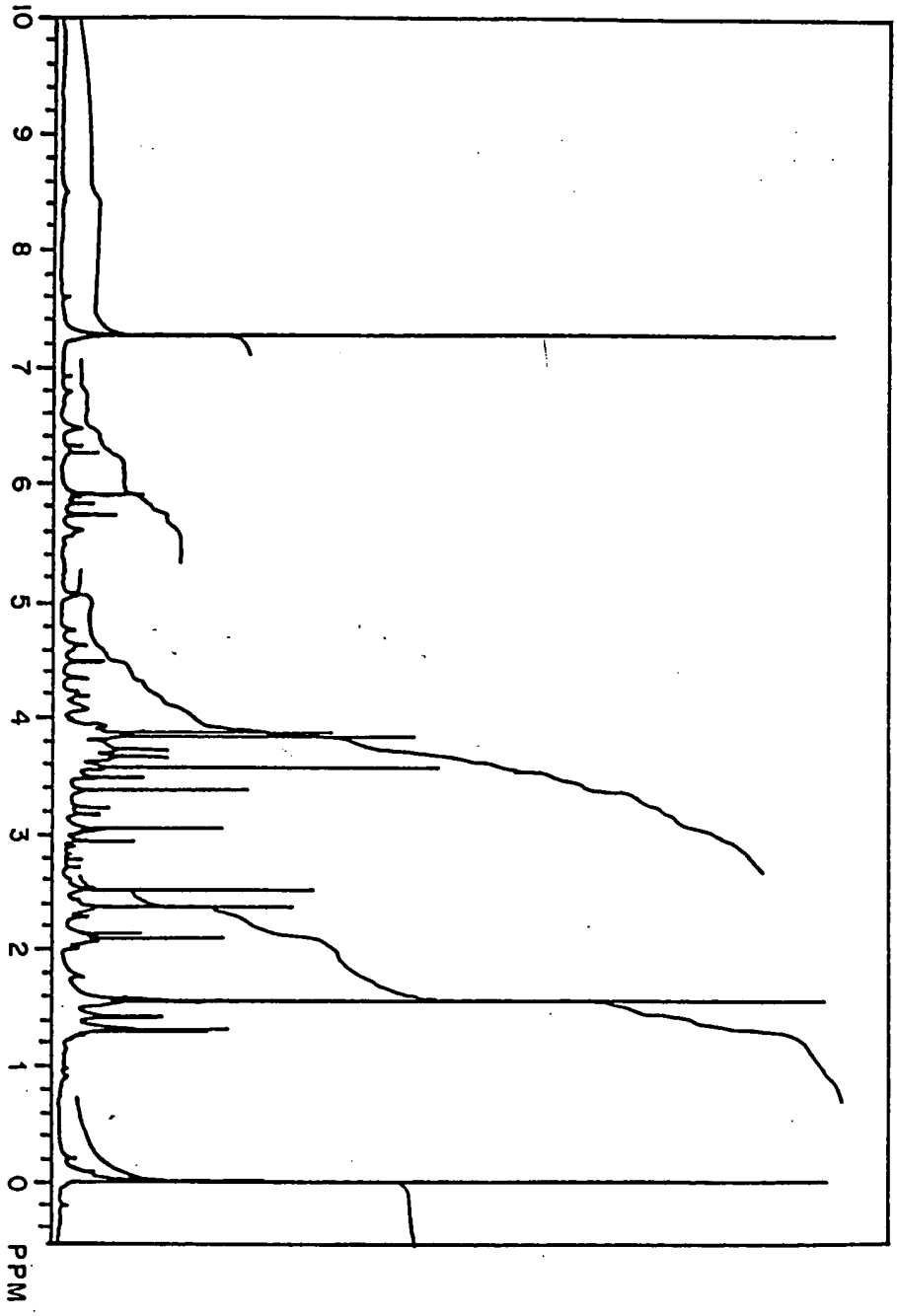

FIG. 1

PROTON MAGNETIC RESONANCE SPECTRUM OF N-ACETYL-LL-E33288 DELTA, I

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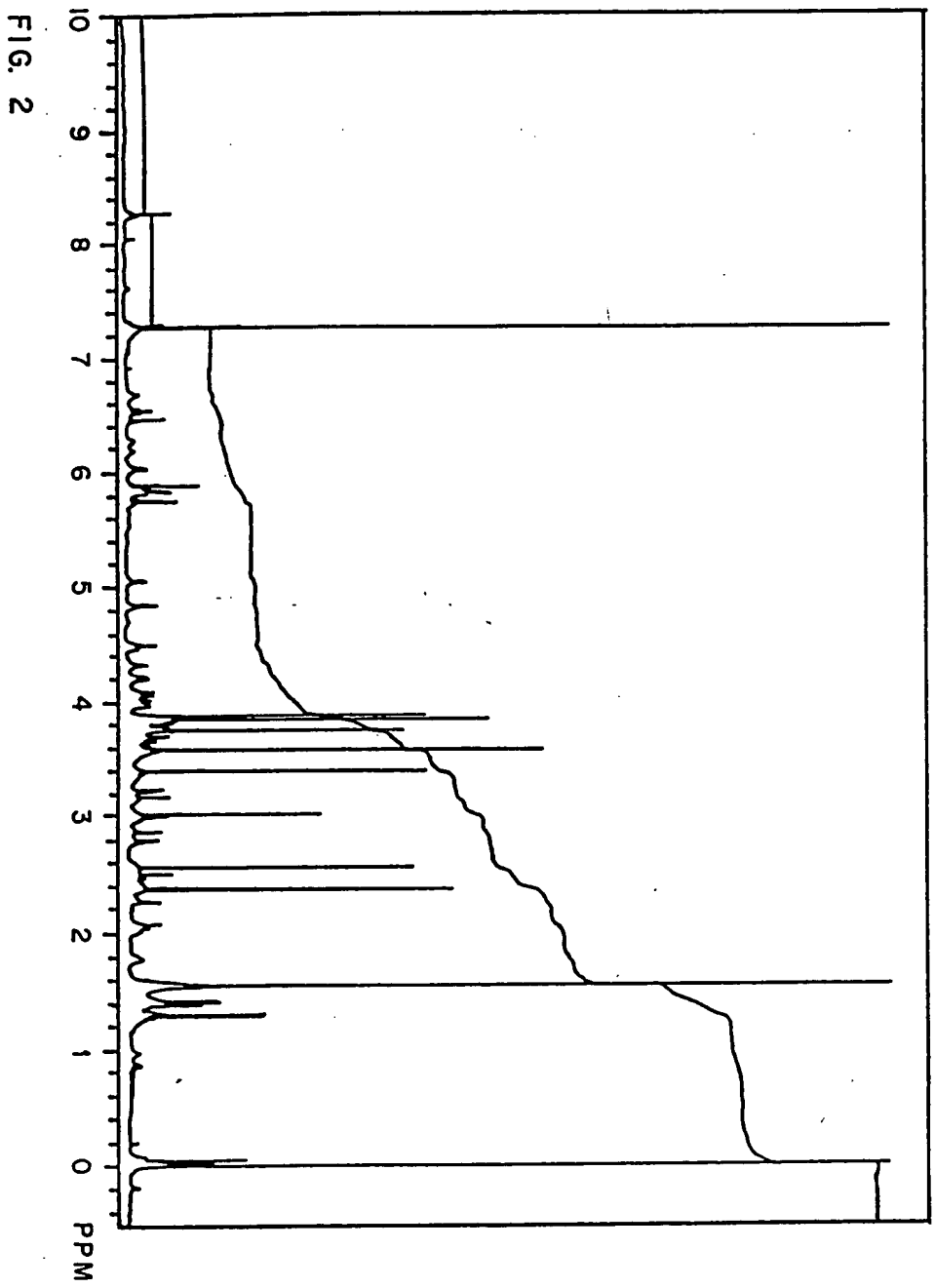

FIG. 2

PROTON MAGNETIC RESONANCE SPECTRUM OF N-FORMYL LL-E33288 DELTA,[1]

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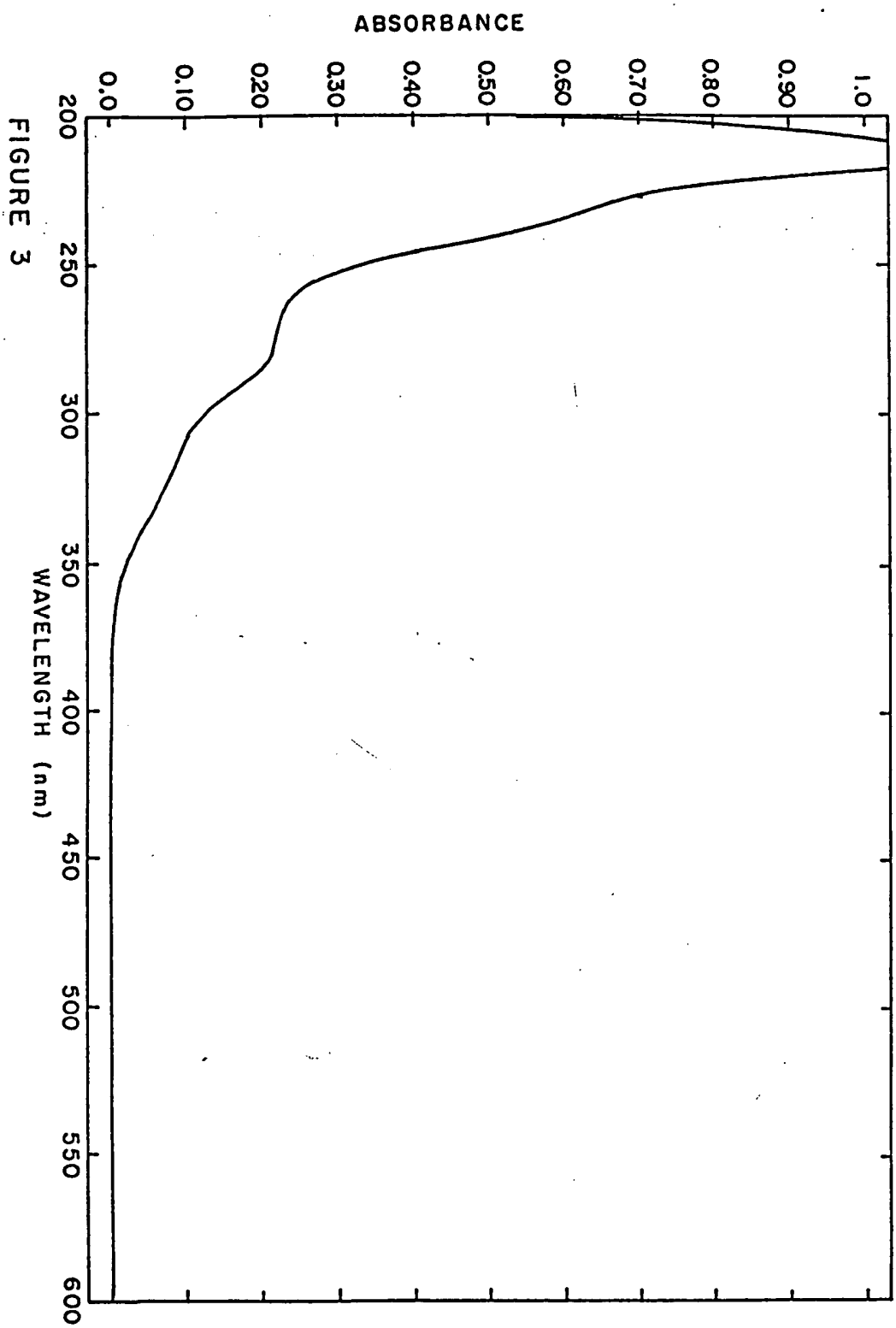

FIGURE 3

ULTRAVIOLET OF N-ACETYL-LL-E33288 γ_1-I

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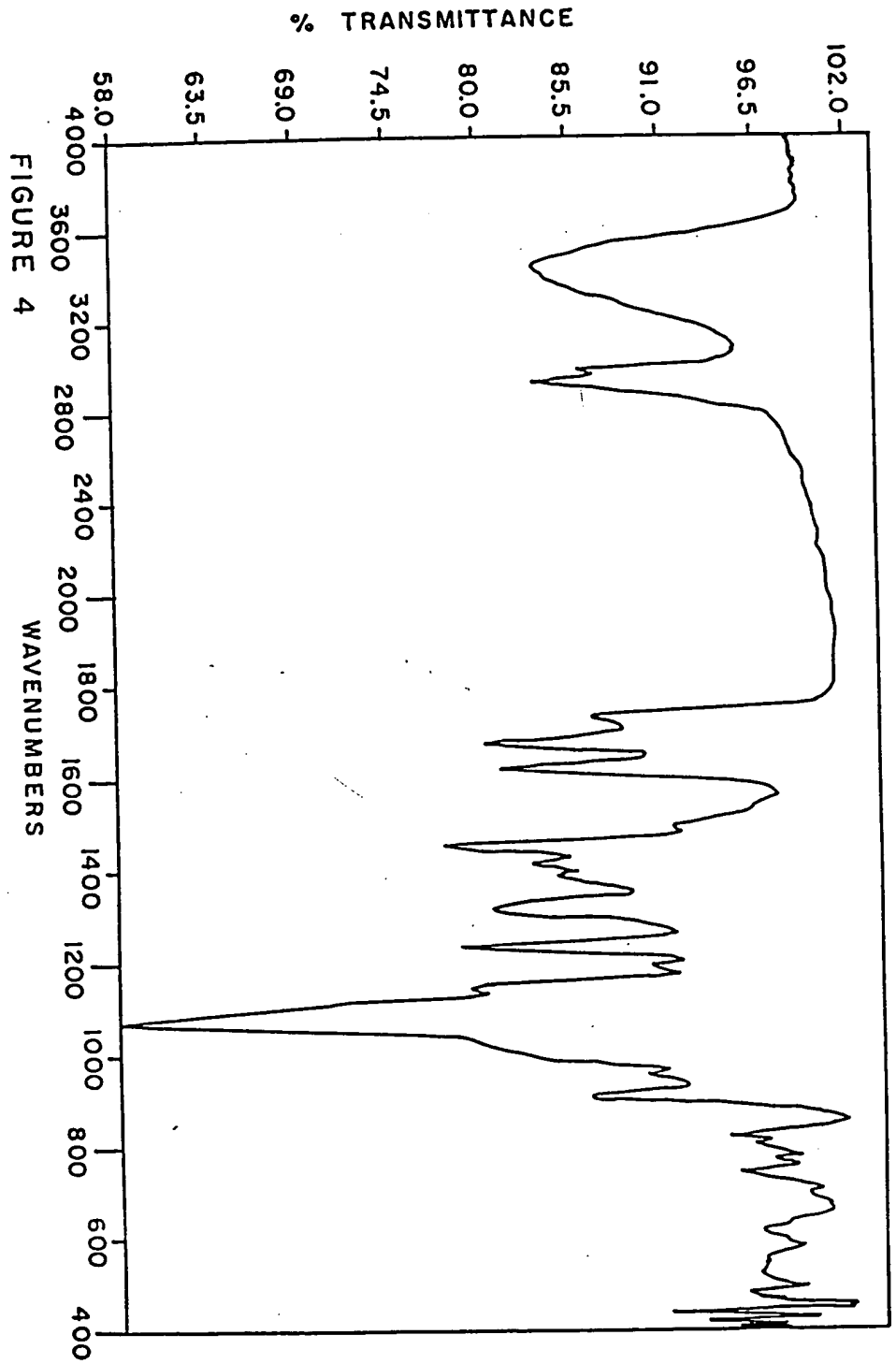

FIGURE 4

INFRARED OF N-ACETYL-LL-E33288 γ_1-I

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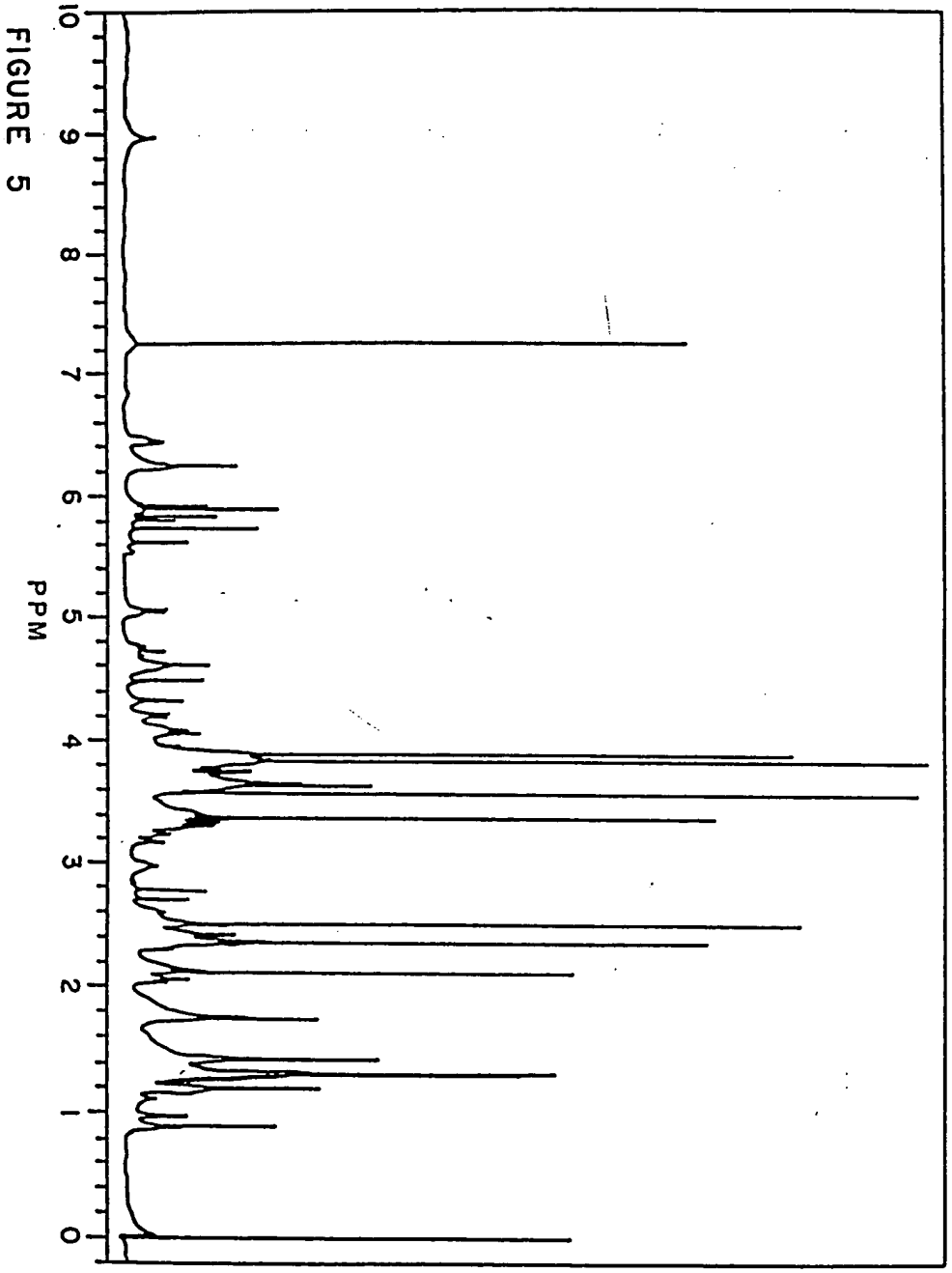

FIGURE 5

PROTON MAGNETIC RESONANCE OF N-ACETYL-LL-E33288γ1-I

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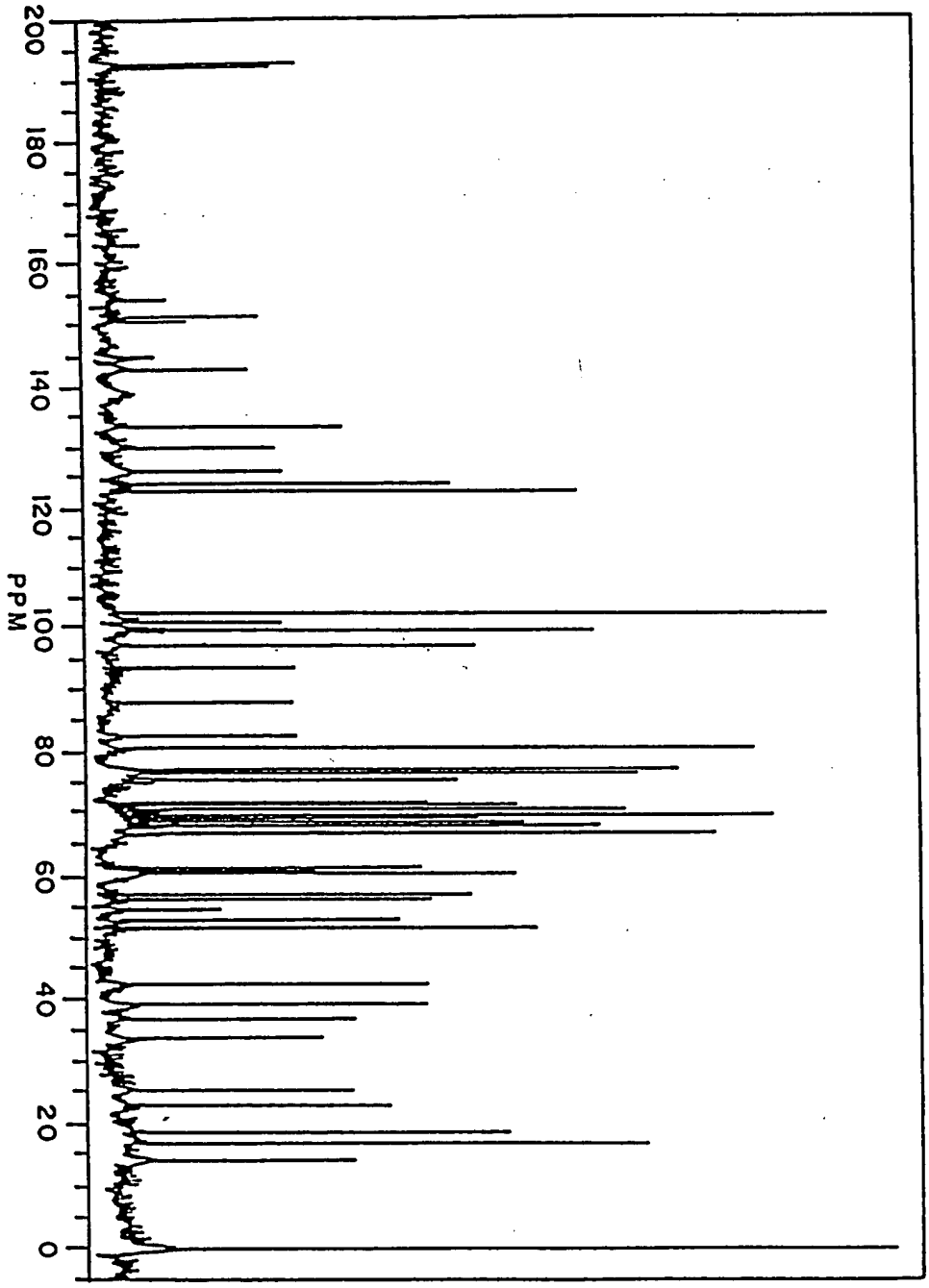
FIGURE 6



CARBON 13 OF N— ACETYL— LL-E33288 γ_1-I

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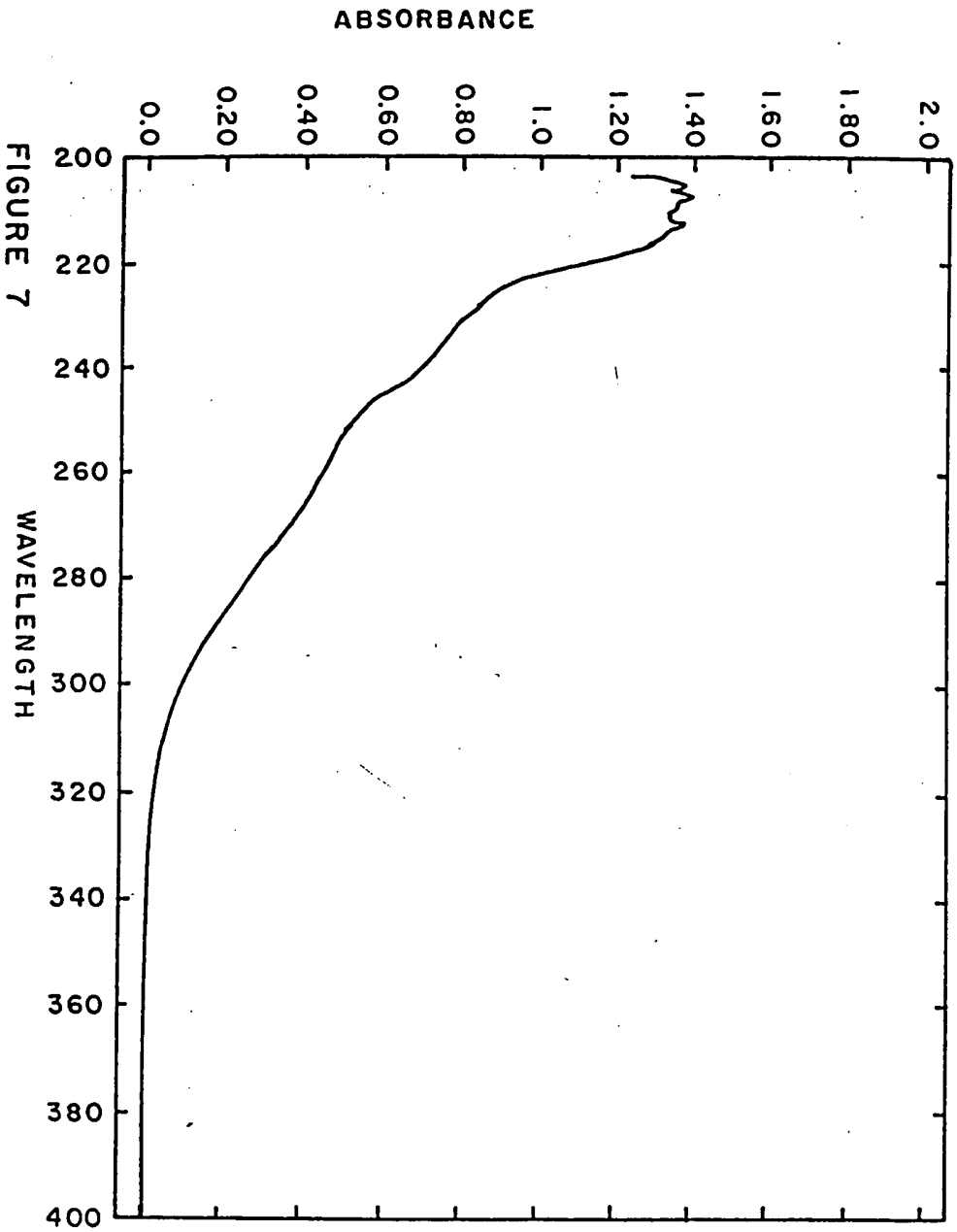

FIGURE 7 THE INFRARED ABSORPTION SPECTRUM OF N-ACETYL-DIHYDRO-LL-E33288 γ'I

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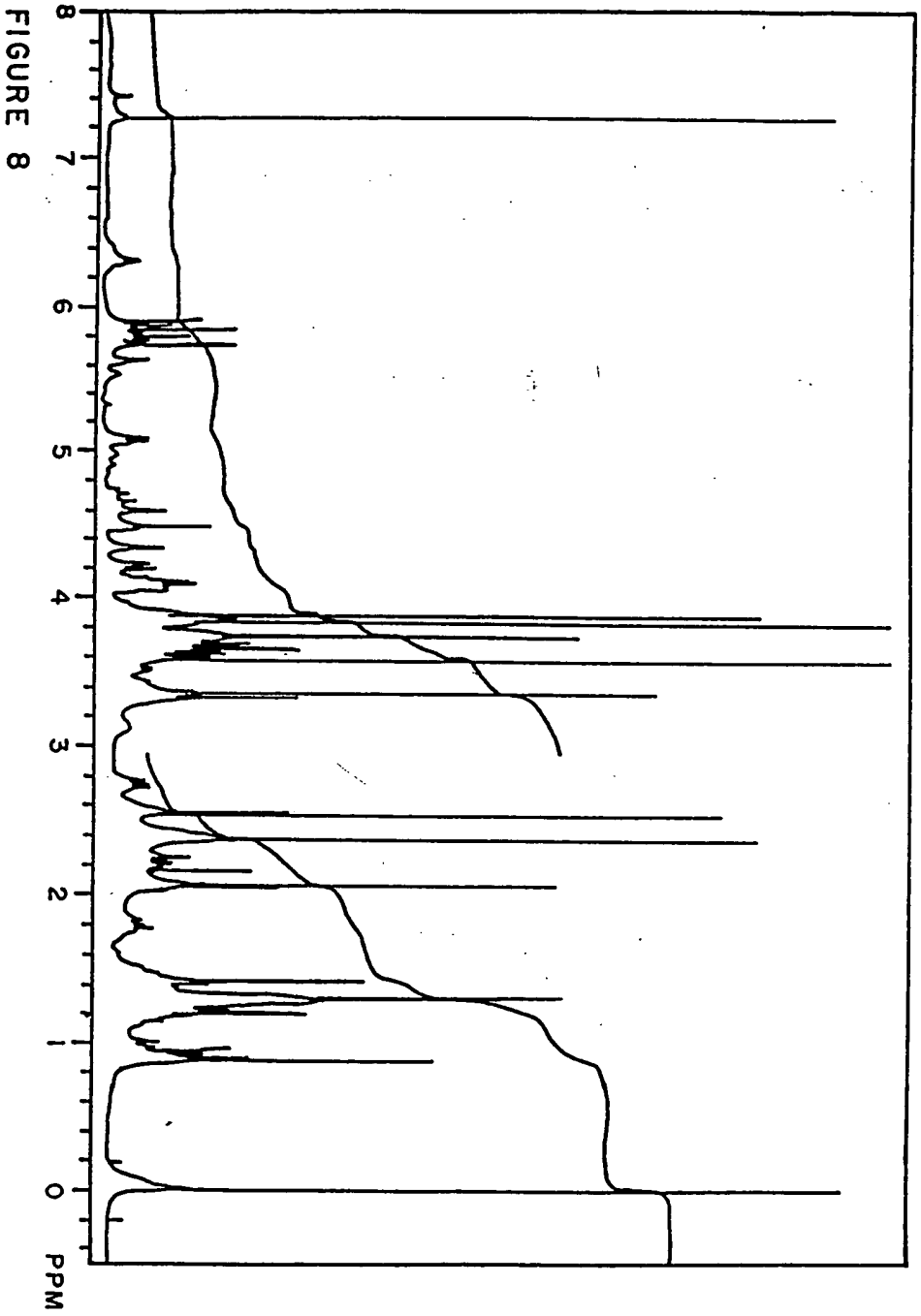

FIGURE 8

THE PROTON MAGNETIC RESONANCE SPECTRUM OF N-ACETYL-DIHYDRO-LL-E33288γ_1

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